

Movie legends.

Grenklo_movieFig2.mov

PxA severely impairs intracellular *Listeria* motility. *Listeria*-infected PtK2 cells were injected with 20 mg/mL PxA and followed by video microscopy. The injection of PxA caused the inhibition of *Listeria* motility that was followed by the rapid disassembly of the actin tails. At later time points, *Listeria* was associated with a fuzzy, phase dense material and their movement was slow, irregular and lacked of directionality.

Grenklo_movieFig3.mov

PxA reduces *Listeria* speed and causes actin tail detachment. MDCK cells stably expressing GFP-actin were infected with *Listeria* and then injected with 5 mg/mL PxA. Shortly after injection, a break appears between the bacterium and its tail, which is followed by the complete depolymerisation of the actin tail. The bacterium remained associated with a cloud of actin filaments.

Grenklo_movieFig4.mov

The ability of PxA to interact with proline-rich regions is essential for its inhibitory action on *Listeria* motility. PtK2 cells infected with wild-type *Listeria* were injected with 5 mg/mL P_{H133S}XA and followed by video microscopy. Before injection, wild-type *Listeria* was associated with actin tails and moved at normal speed, which was not affected by the injection of P_{H133S}XA.

Grenklo_movieFig5.mov

PxA does not inhibit the initiation of *Listeria* actin tails in mouse cytosolic brain extracts. Mouse cytosolic brain extracts were incubated with 10mg/mL PxA for 30 minutes on ice. Afterwards, bacteria were added to the mixture and incubated for 15 minutes at room temperature. In control extracts (left panel), *Listeria* induced the formation of long actin tails and moved at an average speed of $0.5 \pm 0.16 \mu\text{m}/\text{min}$. The presence of PxA did not inhibit the initiation of actin tails although the bacteria were characterised by reduced motility (right panel) and were associated with short actin tails. Note that bacteria occasionally aggregate during centrifugation prior to incubation with the extract. This led to movement diverging from a central point that is clearly visible in right panel.

Grenklo_movieFig2(sup mat).mov

The ability of PxA to interact with proline-rich regions is essential for its inhibitory action on *Listeria* motility. PtK2 cells infected with the *Listeria* mutant ActA5 were injected with 5 mg/mL PxA and followed by video microscopy. The motility of this *Listeria* mutant, which typically induces the formation of short actin tails and moves very slowly, was not affected by the injection of PxA.